Remarks

Status of the Claims

PCT Claims 1-14 are cancelled. Claims 15-33 (set forth in the copy of the marked-up specification annexed hereto) are added and renumbered herein as Claims 1-19. Claims 1-19 are pending.

Amendments to the Specification

The specification is amended herein by submission of a substitute specification under 37 C.F. R. § 1.121(b)(3) to simplify the document for printing or copying. A marked-up copy of the specification pursuant to 37 C.F.R. § 1.125(a) is enclosed. In summary, several headings in the specification have been amended, several headings and paragraphs have been added, and typographical, grammatical and syntactical errors have been corrected to improve the readability thereof. An Abstract is also provided. Support for the substitute specification is found throughout the originally-filed specification.

The amendment on page 21 of the specification to change EP application No. from 02292960 to 02292969 merely corrects a typographical error. EP application No. 02292960 relates to a method for messaging over mobile phone networks. EP application No. 02292969 relates to the production of flavonoid derivatives. Support for the amendment is found in PCT Publication No. WO 05/000831, where the application is cited in the Search Report as EP 1 426 445.

No new matter is added.

Added Claims

PCT Claims 1-14 are cancelled and replaced with new U.S. Claims 15-33 (renumbered as Claims 1-19 as part of the substitute specification). Claims 1-19 are added to correct grammatical and syntactical errors and to place the claims in U.S. format. The subject matter and scope of new Claims 1-19 correspond generally to cancelled PCT Claims 1-14. Support for Claims 1-19 is found throughout the originally-filed specification and in cancelled PCT Claims 1-14. No new matter is added.

Conclusion

The amendments to the specification and claims made herein are proper and entry is respectfully requested. Early examination of the substitute specification is respectfully requested.

Respectfully submitted,

PHILIPPE MOUSSOU *et al.*

December 20, 2005
(Date)

By: Jane E Alexander

Jane E. Alexander
Registration No. 36,014
COGNIS CORPORATION
300 Brookside Avenue
Ambler, PA 19002
Telephone: (215) 628-1474
Facsimile: (215) 628-1345
EMail: JANE.ALEXANDER@COGNIS.COM

JEA/ras

TITLE

Esters Of Flavonoids With ω-Substituted C6-C22 Fatty Acids

Cross-Reference to Related Applications

This application is a 35 U.S.C. § 371 filing of International Application No. PCT/EP2004/006281, filed on June 11, 2004, and which claims priority from European application No. EP 03013899.4, filed on June 20, 2003, the entire disclosures of each application are hereby incorporated by reference.

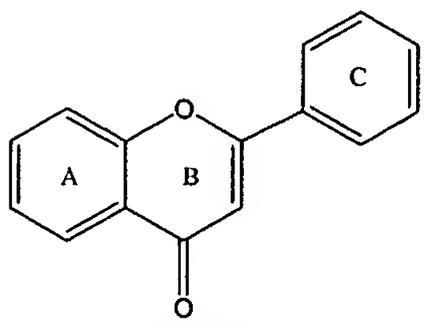
~~Brief description~~ Field of the invention

The invention ~~refers~~ relates generally to esters of flavonoids, and more particularly to ester of flavonoids including ~~such as~~ flavones, flavonols, flavanones, flavanols, flavanolols, isoflavones, anthocyanins, proanthocyanidins, chalcones, aurones and hydroxycoumarins conjugated by an ester bond to a ω-substituted C6 to C22 fatty acid. In addition it ~~refers~~ relates to cosmetic, pharmaceutical formulations and nutritional products comprising these flavonoid derivatives and the use thereof.

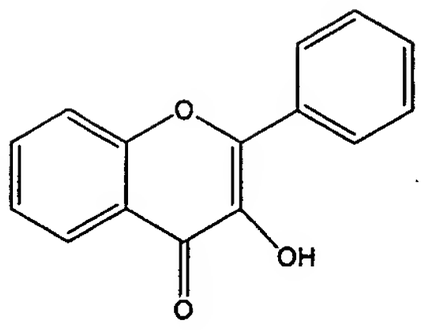
~~State of the art~~

Background Information

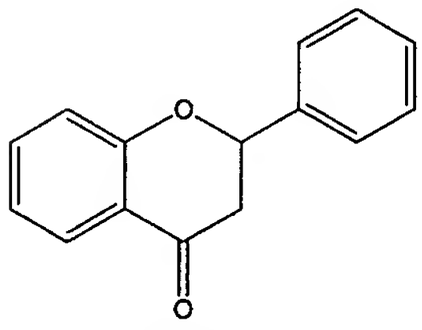
Flavonoids are a class of natural occurring polyphenols in plants. They are benzo-γ-pyron derivatives and can be classified into several groups (flavones, flavonols, flavanones, flavanols, flavanolols, isoflavones, anthocyanins, proanthocyanidins, chalcones, aurones, hydroxycoumarins) according to the presence of different substituents on the rings and the oxidative degree of ring C (figure 1). These flavonoids may also exsist in a glycoside or aglycon form, other modifications such as methylation or acylation of hydroxyl groups increase the diversity of these molecules and their properties.



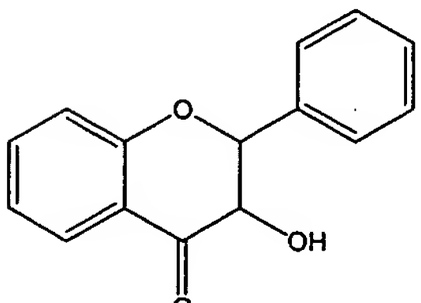
flavone



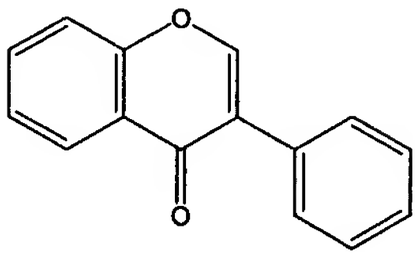
flavonol



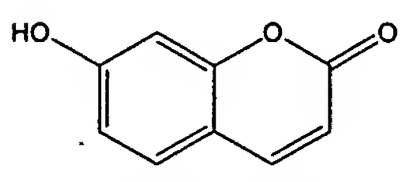
flavanone



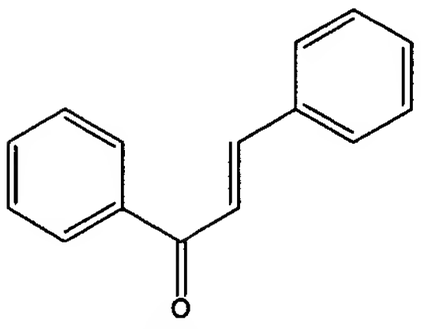
flavanolol



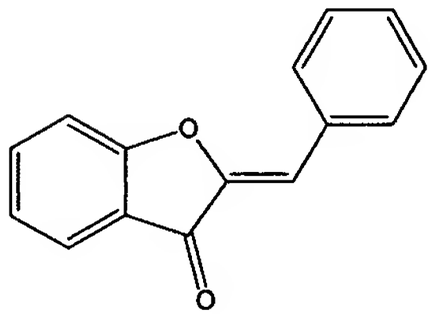
isoflavone



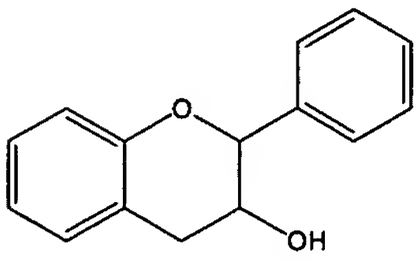
hydroxycoumarin



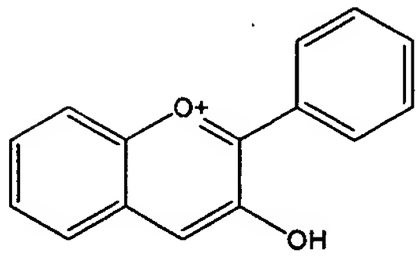
Chalcone



Aurone



flavan-3-ol



Anthocyanin

Figure 1: Different groups of flavonoid derivatives

~~Since~~ For many years, flavonoids ~~are~~ have been known for their biological activities. The main properties are their antioxidant activities and enzyme inhibiting activities. They are already used in cosmetic and pharmaceutical formulations for applications associated to various properties such as anti-erythema, anti-blotchiness, sensitive skin, draining, slimming, anti-wrinkles, stimulation of the extracellular matrix, toning up, skin elasticity, anti-ageing, cardiovascular diseases, veinotonic, inflammation, allergy, antiviral, antibacterial properties, stabilizing or protecting therapeutical agents.

For reasons of their anti-radical activity, combined with their absorption spectrum in the UV range, flavonoids may be of interest to prevent photo-oxidative skin damage. UV radiation is one aspect of environmental stress on the skin. The main UV radiation attacking the skin is in the range of 290 – 320 nm (UVB) reaching the dermis and upper dermis and 320-400 nm (UVA), the most penetrating radiation that affects the dermis. Nuclear or mitochondrial DNA damages, and generation of reactive oxygen species (ROS) which are responsible for lipid and protein damage, are induced by UVA and/or UVB radiation and involve immediate and transient biological responses, for example, inflammation, sunburn, loss of skin elasticity, and delayed and chronic biological responses such as photoaging, or photocarcinogenesis. However, Saija et al. (1998, International Journal of Pharmaceutics, 175, 85), ~~have~~ demonstrated that flavonoids were ineffective in formulations.

Moreover, the application of flavonoids in cosmetic, pharmaceutical preparations and nutrition are limited by their low solubility and stability. The solubility of flavonoids (glycosylated and aglycon) in both aqueous phase and lipophilic phase are low. Thus, it is very difficult to incorporate flavonoids in cosmetic, pharmaceutical or nutraceutic formulations. A second drawback is a poor bioavailaibility of flavonoids. Flavonoids are instable due to the presence of many hydroxyl groups in their structure. They are degraded by light, oxygen or oxidizing agents and high temperature.

To improve the UV-protection properties of flavonoids, combination by acylation or alkylation of flavonoids, particularly tiliroside, with aromatic compounds known for their UV-filter properties – for example dibenzoylmethane derivatives or benzoyl derivatives - have been described in ~~the~~ International application WO 02/069926. The linking of flavonoids to UV-filter molecules increases the stability of UV-filter. In ~~the~~ European application EP 1205475 aglygon flavonoids were also modified with the same UV-filter. These compounds possess the properties of both molecules: the antioxidant and enzyme inhibitor activities of flavonoids and the UV absorption properties of a filter.

In ~~the US patent~~ US Patent No. 4255336 derivatives of cyanidan-3-ol with organic carboxylic acid, carbonic acid, sulphonic acid were described in respect of their activity regarding the prevention of hepatic necrosis and lipoperoxydation. These

compounds could protect the tissue by the inhibition of the degradation of collagen by collagenase.

Different solutions have been proposed to solve the problem of instability of flavonoids such as encapsulation or addition of antioxidants. Another described way for increasing the stability and the lipophilicity of flavonoids is their acylation with fatty acids by chemical or enzymatic ways. In ~~the~~ French patent FR 2706478 therapeutical and cosmetic formulations containing esters of flavanol and procyanolidic oligomers and fatty acid were described. The acylation of phenolic groups has increased the stability of the formulation in respect of color without decreasing the ~~antioxydant~~ antioxidant activity. In FR 2778663 fatty esters of flavonoids were synthesized ~~by~~ chemically ~~way~~. The resulting flavonoid esters were stabilized in preparations and emulsions and their anti-radical activities were preserved. The activity of enzyme inhibition was also increased by the acylation of flavonoids with fatty acids. This is a result of a higher degree of penetration ~~thought~~ through the cell membrane.

In ~~the US patent~~ US Patent No. 5844061 flavonol and procyanolide oligomers were rendered liposoluble and stable by protecting the hydroxyl groups by esterification with fatty acid or aryl acid. The antiradical and antioxidant properties of these esters can be exploited in therapy, cosmetic and dietetic fields.

~~The~~ International patent application WO 00/44757 discloses hydrophilic and lipophilic hesperetin acylated with an organic or inorganic salt of acid or with fatty acid or substituted fatty acid or aromatic acid in order to increase the bioavailability of hesperetin for pharmaceutical application.

The bioavailability of flavonoids may also be improved by increasing their aqueous solubility. Hydrophilic quercetin, apigenin, genistein were obtained by linking a phosphorylated sugar (inositol phosphate) directly or by a short carbon chain (succinate ester). This method increases the aqueous solubility of quercetin due to a linkage with a polar group without diminishing its cytotoxic and antiproliferative activity (WO 96/21440).

In WO 99/63995 the bioavailability of isoflavones was increased by improving their aqueous solubility. This was accomplished by attaching a polar group.

Isoflavones were esterified on an alcohol functionality of aglycon part using a carboxylic acid group or a phosphoric acid group possessing a polar group directly attaching to acid or indirectly linked to a short carbon chain. Succinate, glutarate, adipate and phosphate ester were described as good solubilizers with biological compatibility. Esterified isoflavones can be converted into free isoflavone in biological media by hydrolyzing the ester bond by various enzymes. The esterified isoflavones can be used in nutritional supplements and pharmaceutical preparations as phytoestrogen, antiangiogenic, antioxidant, anticancer, and against ultraviolet skin damage.

Microcapsules of flavonoids have also been obtained by interfacial cross-linking of flavonoids with diacide (FR 2715582). Microcapsules were prepared by mixing an aqueous solution of flavonoid with an organic solution of diacide under vigorous stirring and at elevated pH. The stabilized polyphenol retains its activities.

In ~~the~~ German patent application DE 10019235 glycosylated flavonoids and isoflavones acylated with fatty acid or arylaliphatic acid are claimed for cosmetic and pharmaceutical application.

Dicarboxylic acids, having carboxylic groups at the opposite ends of the hydrocarbon chain, represent an interesting class of fatty acid derivatives with bactericidal properties and enzyme inhibition activity. Moreover the majority of these acids are unable to rapidly ~~across~~ liposome membranes. Azelaic acid is already used as cosmetic and therapeutic agent for bleaching of hair, for inhibiting the activity of protease inducing scales and tyrosinase, as anti-acne, antiaging, and as skin lightening agents and have some effects in certain skin disorders.

Accordingly it is an object of the present invention to provide new molecules that combine the properties of flavonoids and ω-substituted C6 to C22 fatty acids with improved biological properties, chemical and physico-chemical stability. These molecules should protect skin, mucus membranes and scalp from damages by UV-radiation and thereby prevent ageing of the skin.

It is another object of the invention to provide formulations comprising these flavonoid derivatives with improved physico-chemical properties and high bioavailability.

Summary of the Invention

Briefly described, according to an aspect of the invention, a flavonoid ester with a ω-substituted C6 to C22 fatty acid, where the ω-substituted C6 to C22 fatty acid is a saturated or unsaturated, linear or branched aliphatic C6 to C22 - carboxylic acid having one or more polar groups is provided. The flavonoid may be an aglycone or the glycosylated form of a polyphenol selected from a flavone, a flavonol, a flavanone, a flavanol, a flavanolol, an isoflavone, an anthocyanin, a proanthocyanidin, a chalcone, an aurone and a hydroxycoumarin. The polar group may be on the terminal carbon atom of the C6 to C22 – carboxylic acid.

In addition, the polar group of the ω-substituted C6 to C22 fatty acid may be a derivative of a carboxylic acid selected from a carboxylic acid (COOH); an amide ($CONR'_2$ or $CONR'_3{}^+S^-$) wherein R' is a hydrogen atom, a saturated or unsaturated, linear or branched alkyl C1-C6 radical, or an aryl, aralkyl or aralkylene radical and S^- is a counter ion; a COHal where in Hal is a halogen atom; and a COSH. The ω-substituted C6 to C22 fatty acid may also be dicarboxylic, and selected from octanedioic acid, azelaic acid, decandioic acid, dodecandioic acid, hexadecandioic acid and octadecandioic acid. The dicarboxylic acid may also be linked to a flavonoid by an ester bond on one of its carboxylic groups (HOOC-X-C(=O)-O-flavonoid), where X is a saturated or unsaturated, linear or branched alkyl radical ($C_4 - C_{20}$). The ω-substituted C6 to C22 fatty acid may be 11-mercaptoundecanoic acid or thioctic acid, and the polar group of the ω-substituted C6 to C22 fatty acid may be a thiol or an alkylthioalkyl group. The ω-substituted C6 to C22 fatty acid may have two adjacent polar groups selected from diol, dithiol, 1,2-dithiane, 1,3-dithiane and epoxide.

In another aspect of the invention, a nutritional, cosmetic or pharmaceutical composition contains a flavonoid ester described above.

In another aspect of the invention, a nutritional, cosmetic or pharmaceutical composition including liposomes or microcapsules contains a flavonoid ester described above. The nutritional or cosmetic or pharmaceutical composition may contain 0.0001 to 10 wt % of the flavonoid ester.

In another aspect of the invention, the flavonoid ester may be incorporated into a cosmetic preparation as an agent to protect skin and scalp against damage caused

by UV radiation, mitochondrial or nuclear DNA damage caused by UV radiation, and aging, or as an anti-inflammatory and/or soothing and relieving agent.

In another aspect of the invention, the flavonoid ester may be incorporated into a preparation for stimulating the metabolism and the immune defense of human skin, including defense against oxidative or environmental stress or pollutants, for a dermatological anti-inflammatory care preparation, or for a draining, veinotonic or slimming preparation.

The flavonoid ester may be used in the above-desribed preparations in quantities of 0.0001 to 10 wt % based on the final composition. The flavonoid ester may also be present in the preparations in the form of liposomes or microcapsules.

Detailed ~~d~~ D escription of the ~~i~~ I nvention

The present invention relates to flavonoid esters with ω-substituted C6 to C22 fatty acids. In addition it relates to nutritional, cosmetic or pharmaceutical compositions containing these flavonoid esters and compositions wherein these flavonoid esters are incorporated in liposomes or microcapsules.

~~Further on the~~ The invention ~~concerns~~ also relates to the use of flavonoid esters with ω-substituted C6 to C22 fatty acids to protect skin and scalp against damages caused by UV-radiation such as mitochondrial or nuclear DNA damage ~~for~~ from skin aging, to protect against oxidative stress, environmental stress or pollutants, or as an anti-inflammatory agent.

Surprisingly it has been found that the esters of flavonoids with ω-substituted C6 to C22 fatty acids have the property to protect the skin cells against damages caused by UV radiation. As shown in the examples, we have found that the esters of flavonoids according to the invention protect skin cells against UVA and UVB radiation in a more effective manner than the flavonoids alone. Moreover, these esters demonstrated their property to stimulate the GSH metabolism of human skin cells after UVA irradiation, *i.e.*, to stimulate their cellular ~~defences~~ defenses. They have also anti-inflammatory and soothing properties, as demonstrated by the inhibition of released PGE2 after UVB irradiation.

Thereby these flavonoid esters may be used to protect the skin and scalp and/or to fight against UV and sun damages, erythema, sunburn, mitochondrial or nuclear DNA damages, to prevent or fight photo-aging, providing improvement for signs of ageing as skin wrinkles, elasticity ~~loss~~ is lost and a decrease in skin thickness.

They may be used also to protect skin, scalp and/or hair shaft and fight against oxidative or stress damages, to protect skin, scalp and/or hair shaft from environmental stress such as pollutants~~,~~ and chemicals.

They may be used to improve the appearance of the skin with local inflammations or microinflammations. Moreover, they may be used to treat sensitive or irritated skin or scalp, as ~~appeasing~~ a soothing and anti-itching agent.

Since the flavonoid esters still exhibit the activities of the pure flavonoids the invention allows also their use as anti-free radicals, anti-oxidant, anti-blotchiness agents, for draining treatment, for slimming treatment, for anti-wrinkle treatment, as stimulator of the synthesis of elastin and other extracellular matrix elements, in toning up compositions. They may be used also in compositions for applications related to cardiovascular diseases, veinotonic effect, inflammation disorders, allergy, antiviral and antibacterial properties, stabilizing or protecting therapeutical agents.

The disclosed flavonoid esters show a very good chemical stability. Flavonoid esters with ω-substituted C6 to C22 fatty acids also have a better solubility in lipophilic vehicles, and so they can be easily incorporated in cosmetic, dermatological, pharmaceutical formulations and as nutrional supplements.

Compared to compositions disclosed in ~~the~~ International patent application WO 99/63995 the bioavailability of isoflavones was further increased by improving their lipophilic solubility. This was accomplished by attaching not only a polar group, but inserting a C6 to C 22 chain of the fatty acid. Flavonoid esters with ω-substituted C6 to C22 fatty acids can directly be dissolved in the oil phase of the formulations, or totally or partially incorporated in liposomes or microcapsules.

The incorporation in liposomes or microcapsules has the advantage that the release of the active flavonoid esters can be controlled. Especially the disclosed lipophilic flavonoid derivatives are easily incorporated in delivery systems for controlled release. These delivery systems have a very good physico-chemical stability due to the solubility profile of the special flavonoid esters, which also results in an approved bioavailability.

The effective quantity of the disclosed flavonoid esters in formulations is 0.0001 to 10 wt %, preferably 0.001 to 5 wt %, most preferably 0.01 to 2 wt % based on the final composition.

Flavonoids

The term flavonoid represents an aglycone or glycosylated form of the following class of polyphenols chosen from the group consisting of flavones, flavonols, flavanones, flavanols, flavanolols, isoflavones, anthocyanins, proanthocyanidins, chalcones, aurones, hydroxycoumarins. Preferably the glycosylated form is chosen.

Preferably the flavonoids are selected from the group consisting of aglycones or the glycosylated form of kampferol, phloretin, apigenin, luteolin, apigenin, quercetin, hesperetin, naringenin, cyanidin, gossypetin, genistein, daidzein, catechin, epicatechin, fisetin, liquiritigenin and esculetin. More preferably, the flavonoids are selected from the group consisting of the glycosylated forms of quercetin as rutin, glycosylated form of hesperetin as hesperidin, glycosylated form of naringenin as naringin, and glycosylated form of esculetin as esculin.

ω-substituted C6 to C22 fatty acids

The term ω-substituted C6 to C22 fatty acid represents a saturated or unsaturated, linear or branched aliphatic carboxylic acid with 6 to 22 carbon atoms having one or more polar group(s) – besides the carboxylic acid group - on carbon atoms anywhere in the chain, preferably at the terminal carbon atom. Preferably these fatty acids have 8 to 18 carbon atoms.

Thise polar group may be:

(a) a derivative of carboxylic acid chosen from the group consisting of a carboxylic acid COOH; an amide $CONR'_2$ or $CONR'_3{}^+S^-$ wherein R' is a hydrogen atom, a saturated or unsaturated, linear or branched alkyl C1-C6 radical, or an aryl, aralkyl or aralkylene radical and S^- a counterion; a COHal wherein Hal is a halogen atom and a COSH.
Examples of these ω-substituted C6 to C22 fatty acid group are octanedioic acid, azelaic acid, decandioic acid, dodecandioic acid, hexadecandioic acid, octadecandioic acid.

(b) a thiol or an alkylthioalkyl group such as 11-mercaptoundecanoic acid,

(c) a primary, secondary, tertiary amine or a quaternium salt of hydrogen atom, a saturated or unsaturated, linear or branched alkyl C1-C6 radical, or an aryl, aralkyl or aralkylene radical such as 11-aminoundecanoic acid,

(d) an halogen atom,

(e) a nitro NO_2 group,

(f) an organic or inorganic phosphoric or sulphuric acid,

(g) a hydroxyl group or an alkoxyalkyl group, [[,]] such as 16-hydroxyhexadecanoic acid, and 12-hydroxystearic acid.

The most preferred derivatives are the derivatives of carboxylic acids (group (a)), especially dicarboxylic acids.

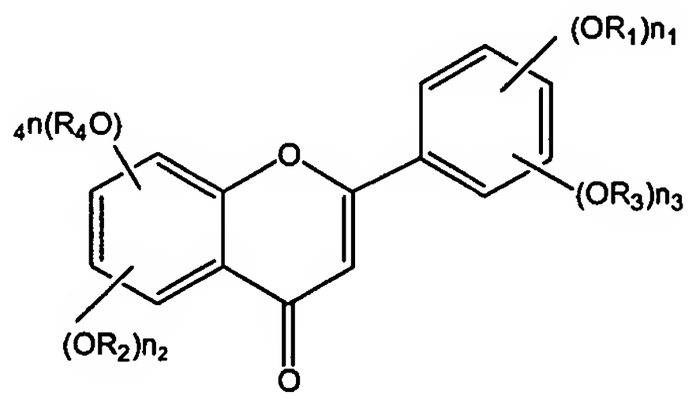
The ω-substituted C6 to C22 fatty acid is also represented by a di-carboxylic acids linked to a flavonoid by an ester bond on one of its carboxylic group, i.e. HOOC-X-C(=O)-O-Flavonoid, wherein X is a saturated or unsaturated, linear or branched alkyl radical ($C_4 - C_{20}$).

The ω-substituted C6 to C22 fatty acid is also represented by a saturated or unsaturated, linear or branched aliphatic chain (C6-C22) having two adjacent polar groups which are diol, dithiol, 1,2 and 1,3 dithiane, and epoxide, such as thioctic acid.

Flavonoid esters of the invention

The esters of flavonoids with ω-substituted C6 to C22 fatty acids of the invention ~~are characterized in that they~~ correspond to formulas (I) to (X):

Flavone (I) :



(I)

wherein:

(h) the (OR_1), (OR_2), (OR_3) and (OR_4) groups are anywhere on the ring,

(i) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical ($C_1 - C_6$), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atoms, a monosaccharide or an oligosaccharide,

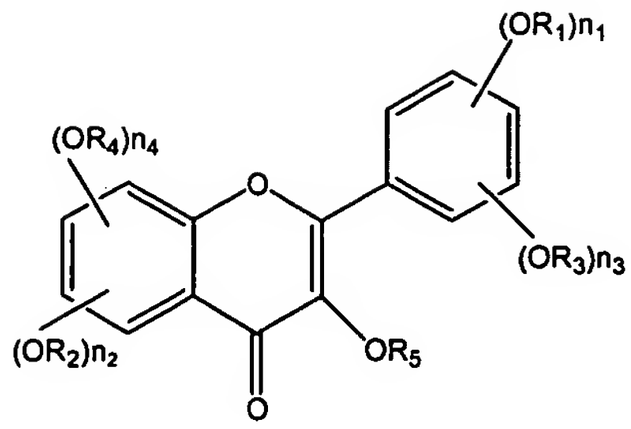
(j) R_3 and R_4 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

(k) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_2$ does not exceed 5 [[.]] , and

(l) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 4, and the sum $n_3 + n_4$ does not exceed 4.

Examples of flavones are apigenin, luteolol as aglycon form and their glycosylated forms such as diosmin, orientin, saponarin, and shaftoside.

The monosaccharide may be preferably substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. The oligosaccharide may be preferably the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.



Flavonol (II) :

(II)

wherein:

(m)the (OR_1), (OR_2), (OR_3) and (OR_4) groups ~~were~~ are anywhere on the ring,

(n) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical $(C_1 - C_6)$, a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atoms, a monosaccharide or an oligosaccharide,

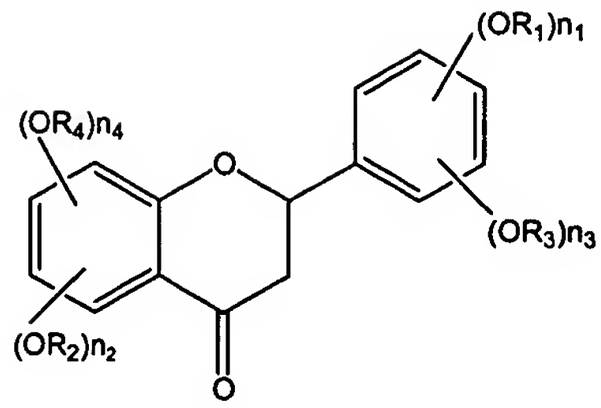
(o) R_3, R_4 and R_5 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

(p) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_3$ does not exceed 5 [[.]] , and

(q) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 4, and the sum $n_2 + n_4$ does not exceed 4.

Examples of flavonol are kaempferol, quercetin, rhamnetin as aglycon form and their glycosylated form as rutin, quercitrin, hyperoside, and isoquercitrin.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.



Flavanone (III):

(III)

wherein:

(r) the (OR_1), (OR_2), (OR_3) and (OR_4) groups ~~were~~ are anywhere on the ring,

(s) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical ($C_1 - C_6$), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atoms, a monosaccharide or an oligosaccharide,

(t) R_3, R_4 and R_5 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

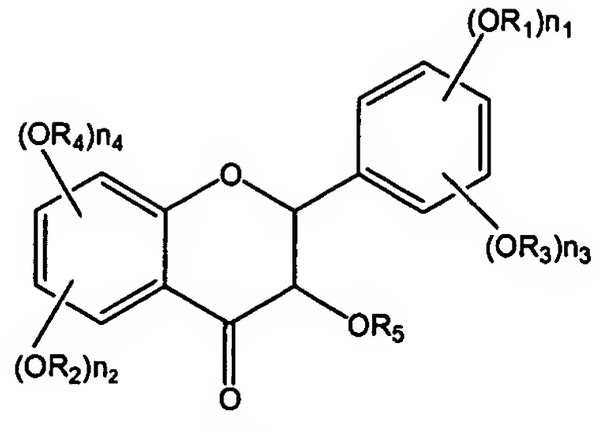
(u) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_3$ does not exceed 5 [[.]] , and

(v) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 4, and the sum $n_2 + n_4$ does not exceed 4.

Examples of flavanon are naringenin, eriodictyol, hesperetin, eucalyptin, cirsimaritin, cajaflavanon, hinokiklavon, amentaflavon, bilobetol as aglycon form and their glycosylated form such as hesperidin, neohesperidin, prunin, and naringin.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.

Flavonolol (IV):



(IV)

wherein:

(w) the (OR_1), (OR_2), (OR_3) and (OR_4) groups ~~were~~ are anywhere on the ring,

(x) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical ($C_1 - C_6$), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atoms, a monosaccharide or an oligosaccharide,

(y) R_3, R_4 and R_5 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

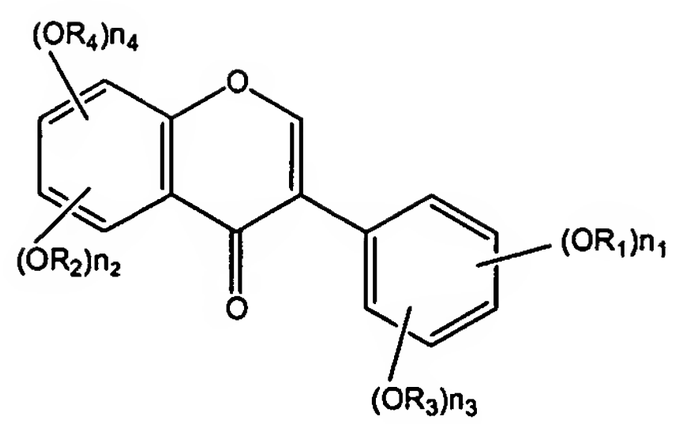
(z) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_3$ does not exceed 5 [[.]] , and

(aa) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 4, and the sum $n_2 + n_4$ does not exceed 4.

Examples of flavanolol (also named dihydroflavonol) are fustin, garbanzol, taxifolin, 6-methoxytaxifolin, dihydrokaempferol, dihydrorobinetin as aglycon form and their glycosylated form.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be a sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.

Isoflavone (V) :



(V)

wherein:

(bb) the (OR_1), (OR_2), (OR_3) and (OR_4) groups ~~were~~ are anywhere on the ring,

(cc) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical ($C_1 - C_6$), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atoms, a monosaccharide or an oligosaccharide,

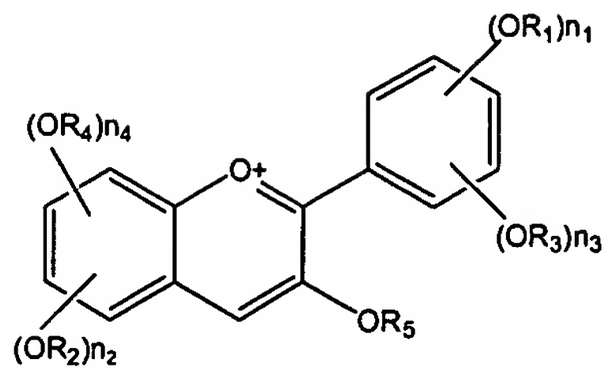
(dd) R_3 and R_4 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

(ee) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_3$ does not exceed 5 [[.]] , and

(ff) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 4, and the sum $n_2 + n_4$ does not exceed 4.

Examples of isoflavonoids are daidzein, genistein, biochanin A, formonetin, cajanin, prunetin, irigenin, luteone as aglycon form and their glycosylated form as daidzin, genistin, iridin, and puerarin.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.



Anthocyanin (VI) :

(VI)

wherein:

(gg) the (OR_1), (OR_2), (OR_3) and (OR_4) groups ~~were~~ are anywhere on the ring,

(hh) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical ($C_1 - C_6$), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atom, a monosaccharide or an oligosaccharide,

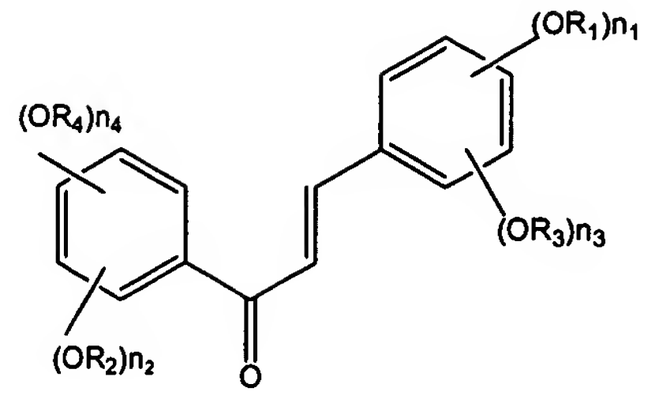
(ii) R_3, R_4 and R_5 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

(jj) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_3$ does not exceed 5 [[.]] , and

(kk) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 4, and the sum $n_2 + n_4$ does not exceed 4.

Examples of anthocyanins are cyanidin, 6-hydroxycyanidin, pelargonidin, okanin, malvidin as aglycon form and their glycosylated form as cyanidin-3-*O*-galactoside, cyanidin-3-*O*-rutinoside, pelargonidin, and malvin.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.



Chalcone (VII) :

(VII)

wherein:

(ll) the (OR_1), (OR_2), (OR_3) and (OR_4) groups ~~were~~ are anywhere on the ring,

(mm) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical ($C_1 - C_6$), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atom, a monosaccharide or an oligosaccharide,

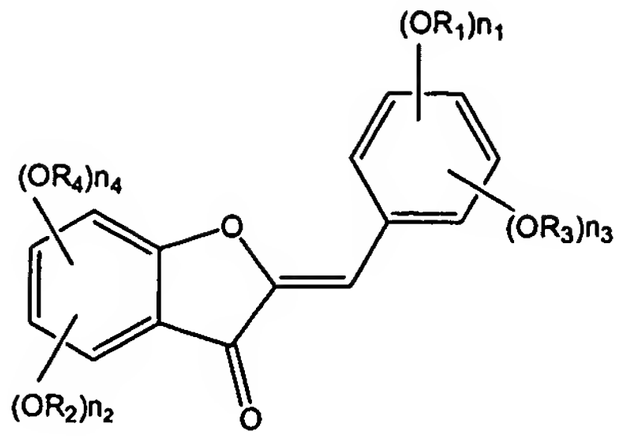
(nn) R_3 and R_4 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

(oo) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_3$ does not exceed 5 [[.]] , and

(pp) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_2 + n_4$ does not exceed 5.

Examples of chalcones are davidigenin, phloretin, isoliquiritigenin as aglycon form and their glycosylated form as phloridzin, and glycyphyllin.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.



Aurone (VIII) :

(VIII)

wherein:

(qq) the (OR_1), (OR_2), (OR_3) and (OR_4) groups ~~were~~ are anywhere on the ring,

(rr) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical ($C_1 - C_6$), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atom, a monosaccharide or an oligosaccharide,

(ss) R_3 and R_4 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

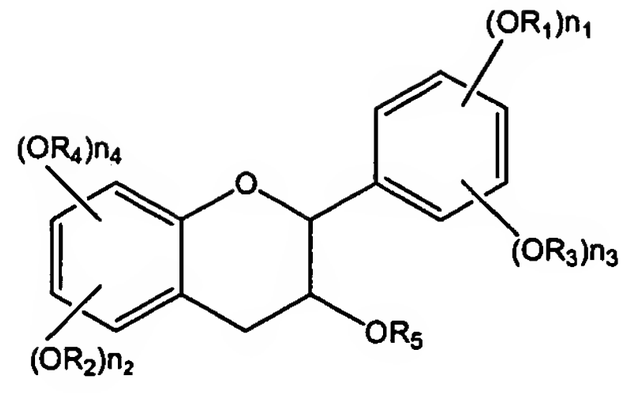
(tt) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_3$ does not exceed 5 [[.]] , and

(uu) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 4, and the sum $n_2 + n_4$ does not exceed 4.

Examples of aurones are aureusidin, sulphuretin, hispidol as aglycon form and their glycosylated form as 6-glucoside-hispidol.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.

Flavanol (IX) :



(IX)

wherein:

(vv) the (OR_1), (OR_2), (OR_3) and (OR_4) groups ~~were~~ are anywhere on the ring,

(ww) R_1 and R_2 are identical to or different from each other and represent a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical ($C_1 - C_6$), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atom, a monosaccharide or an oligosaccharide,

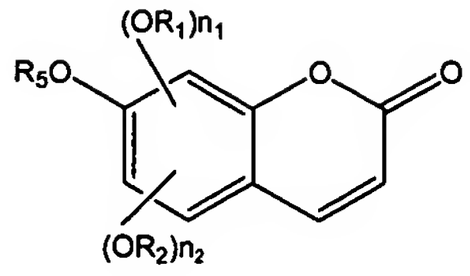
(xx) R_3, R_4 and R_5 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] ,

(yy) n_1 and n_3 are identical to or different from each other, are numbers from 0 to 5, and the sum $n_1 + n_3$ does not exceed 5 [[.]] , and

(zz) n_2 and n_4 are identical to or different from each other, are numbers from 0 to 4, and the sum $n_2 + n_4$ does not exceed 4.

Examples of flavanol (flavan-3-ols) are catechin, epicatechin, fisetinidol as aglycon form and their glycosylated form as catechin-7-*O*-xyloside, cyanidin-3-*O*-rutinoside, pelargonidin, and malvin.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.



Hydroxycoumarin (X) :

(X)

wherein:

(aaa) the (OR_1) and (OR_2) groups ~~were~~ are anywhere on the ring,

(bbb) R_1 represents a hydrogen atom, a saturated or unsaturated, linear or branched alkyl radical (C_1 – C_6), a saturated or unsaturated, linear or branched acyl group with 1 to 6 carbon atom, a monosaccharide or an oligosaccharide,

(ccc) R_2 and R_5 are identical to or different from each other and comprise a ω-substituted acyl group, or a monosaccharide or an oligosaccharide having at least one or more ω-substituted acyl groups, preferably from 1 to 6 acyl groups and more preferably from 1 to 3 acyl groups [[.]] , and

(ddd) n_1 and n_2 are identical to or different from each other, are numbers from 0 to 3, and the sum $n_1 + n_2$ does not exceed 3.

Examples of hydroxycoumarins are esculetin, umbelliferone, scopoletin, fraxetin as aglycon form and their glycosylated form as esculin, cichoriine, and fraxin.

Preferably the monosaccharide may be substituted or unsubstituted glucose, rhamnose, galactose, arabinose, and xylose. Preferably the oligosaccharide may be the sugar moiety of the following flavonoids: tiliroside, orientin, schaftoside, saponarine, rutin, hesperidin, and diosmin or a polymer of one or more monosaccharide(s) previously described.

Preparation of the flavonoid esters

The flavonoid esters according to the invention may be synthesized using known acylation processes from the state of the art. The acylation can be performed using an enzymatic process as described in the recently filed patent application no. EP ~~02292960.9~~ 02292969.9 (Cognis France). The esters can also been obtained by chemical acylation methods. Chemical acylation agent may be chosen among acids of formula RCOOH, the halogen derivatives of these acids RCOHal, anhydrides of formula RCOOCR or esters of formula RCOOR' wherein R' is a C1-C6 alkyl group, in anhydric appropriate solvent under inert atmosphere. Appropriate solvents may be chosen from the group consisting of toluene, pyridine, chloroform, tetrahydrofurane and acetone.

Examples

Example 1: Synthesis of ester of rutin with octadecandioic acid

This reaction was carried out in a 250 ml batch reactor. Rutin (0.85 g, 1.4 mmol) and octadecandioic acid (0.97 g, 3.1 mmol) were dissolved in 250 ml *tert*-amyl alcohol. The medium was heated at 60°C under vacuum (170 mbar). The formed vapor was condensed and recycled to the reactor throught a column filled with molecular sieves (50 g). This procedure allowed a low water level (< 100 mM) in the reactor after 21 h. 2.5 g of the lipase of *Candida antarctica* (Novozym 435), a lipase immobilized on a macroporous acrylic resin with an activity of 7000 PLUg-1 (Propyl Laurate Synthesis), was then added.

After 70 h the enzyme was recovered by filtration. The medium was then concentrated by evaporation of solvent. To eliminate the residual substrates, two systems of extraction were used. A mixture of acetonitrile / heptane (3/5 v/v) is used to remove the palmitic acid, while the separation of rutin was carried out by an extraction with water / heptane (2/3 v/v).

The ^{1}H NMR of the ester obtained was:
^{1}H NMR : (400 MHz, DMSO d_6) : 0.76 (d, 3H), 1.2 (m, 24H), 1.44 (m, 4H), 2.17 (m, 4H), 3.1-3.5 (broad, 8H), 3.7 (d, 1H), 4.45 (s, 1H), 4.65 (t, 1H), 5.44 (d, 1H), 6.19 (d, 1H), 6.36 (d, 1H), 6.83 (d, 1H), 7.5 (m, 2H) ppm.

Example 2: Synthesis of ester of rutin with hexadecandioic acid

The acylation of rutin (0.8 g, 1.3 mmol) with hexadecandioic acid (0.98 g, 3.4 mmol) was carried out as described in example 1.
After 63 hours reaction time the same procedure of purification by liquid-liquid extraction as described in example 1 allowed the recovery of rutin hexadecandioate.

The ^{1}H NMR of the ester obtained was:
^{1}H NMR : (400 MHz, DMSO d_6) : δ 0.75 (d, 3H), 1.2 (m, 22H), 1.45 (m, 4H), 2.16 (m, 4H), 3.1-3.7 (broad, 11H), 4.45 (s, 1H), 4.64 (t, 1H), 5.43 (d, 1H), 6.18 (d, 1H), 6.36 (d, 1H), 6.84 (d, 1H), 7.50 (m, 2H), 12.6 (s, 1H, OH) ppm.

Example 3: Synthesis of ester of rutin with azelaic acid

The acylation of rutin (0.8 g, 1.3 mmol) with azelaic acid (0.58 g, 3.1 mmol) was carried out as described in example 1.
After 55 hours reaction time the enzyme was filtered. The medium was then concentrated by evaporation of solvent. The ester was recovered by two systems of extraction. A mixture of water/heptane (2/3 v/v) was used to removed azelaic acid, the recovery of the ester was carried out by extraction with ethyl acetate.

The ^{1}H NMR of the ester obtained was:
^{1}H NMR : (400 MHz, DMSO d_6) : δ 0.75 (d, 3H), 1.24 (m, 12H), 1.48 (m, 8H), 2.20 (m, 8H), 3.15-3.50 (broad, 8H), 3.68 (d, 1H), 4.46 (s, 1H), 4.65 (t, 1H), 5.43 (d, 1H), 6.19 (d, 1H), 6.37 (d, 1H), 6.84 (d, 1H), 7.50 (m, 2H), 12.6 (s, 1H, C_5-OH) ppm

Example 4: Synthesis of ester of rutin with 11-mercaptoundecanoic acid

The acylation of rutin (0.7 g, 1.2 mmol) with 11-mercaptoundecanoic acid (0.7 g, 3.1 mmol) was carried out as described in example 1.

After 64 hours of reaction time the enzyme was filtered. The solvent was then evaporated and the product was dissolved in methanol. The ester is recovered by two systems of extraction. A mixture of water/heptane (2/3 v/v) is used to remove acid, the recovery of the ester was carried out by extraction with dichloromethane.

The ^{1}H NMR of the ester obtained was:

^{1}H NMR : (400MHZ, DMSO d_6) : δ 0.76 (d, 3H), 1.04 (d, 1H), 1.2 (m, 24H), 1.5 (m, 4H), 1.6 (m, 2H), 2.15 (m, 2H), 2.28 (m, 1H), 2.50 (m, 1H), 2.68 (m, 2H), 3.1-3.9 (broad), 4.45 (s, 1H), 4.55 (m, 1H), 4.65 (t, 1H), 5.07 (d, 1H), 5.12 (d, 1H), 5.28 (d, 1H), 5.44 (d, 1H), 6.2 (s, 1H), 6.37 (s, 1H), 6.84 (d, 1H), 7.46 (m, 2H)

Example 5: Acylation of naringin with octadecandioic acid

The acylation of naringin (0.59 g, 1 mmol) with octadecandioic acid (0.98 g, 3.1 mmol) was carried out as described in example 1.

After 50h reaction time the same procedure of purification by extraction as described in example 1 allowed the recovery of the ester.

Example 6: Synthesis of ester of esculin with octadecandioic acid

The acylation of esculin (0.42 g, 1.2 mmol) with octadecandioic acid (0.97 g, 3.1 mmol) was carried out as described in example 1.

After 50 h reaction time the same procedure of purification by extraction as described in example 1 allowed the recovery of ester.

The structure was confirmed by ^{1}H NMR:

^{1}H NMR : (400 MHz, DMSO d_6) : 1.2 (m, 24H), 1.5 (m, 4H), 2.2 (m, 4H)3.15-3.55 (broad, 2H), 3.61 (t, 1H), 4.11 (dd, 1H), 4.34 (dd, 1H), 4.84 (d, 1H), 6.2 (d, 1H), 6.8 (s, 1H), 7.3 (s, 1H), 7.83 (d, 1H) ppm.

Example 7: Synthesis of ester of esculin with thioctic acid

The acylation of esculin (0.87 g, 2.5 mmol) with thioctic acid (1.23 g, 6 mmol) was carried out as described in example 1.

After 70 hours reaction time the enzyme was filtered. The medium was then concentrated by evaporation of solvent. The ester was recovered by two systems of extraction. A mixture of water/heptane/acetonitrile (2/3/0.4 v/v/v) was used to remove thioctic acid, the recovery of ester was carried out by extraction with dichloromethane.

The structure was confirmed by ^{1}H NMR.

^{1}H NMR : (400 MHz, DMSO d_6): 1.2-1.9 (broad, 8H), 2.1-2.4(broad, 4H), 3.2 (m, 2H), 3.5 (m, 1H), 3.7 (m, 1H), 4.12 (dd, 1H), 4.35 (d, 1H), 4.85 (d, 1H), 5.23 (d, 1H), 5.33 (d, 1H), 6.26(d, 1H), 6.84 (s, 1H), 7.33 (s, 1H), 7.86 (d, 1H) ppm.

Example 8 - UVA cytophotoprotection, anti-oxidative effect

The cytoprotection against UVA irradiation has been evaluated by a test on human fibroblasts because UVA radiation penetrates through the epidermis until the dermis where it induces oxidative stress, mainly by activation of photosensitising biological components, which catalyse the formation of ROS like anion superoxide, hydrogen peroxide and singlet oxygen, and lipoperoxydation of the cell membrane. These oxidative stress effects are evaluated in vitro due to measuring of the level of released MDA (malondialdehyde) and of intracellular GSH (reduced glutathion) (Morlière P., Moisan A., Santus R., Huppe G., Mazière J.C., Dubertret L.: UV-A induced lipid peroxydation in cultured human fibroblasts Biochim. Biophys. Acta (1991) 1084, 3:261-269).

The lipoperoxides formed after UVA irradiation undergo a decay into malondialdehyde which can form cross-links between many biological molecules like proteins with inhibition of enzymes and nucleic bases with risk of mutagenesis. ~~The~~ Glutathione (GSH) is a peptide produced by the cells to protect them from oxidative stress or certain pollutants like mercury or lead. An increase in the GSH level enhances the activity of glutathion-S-transferase, a detoxification

enzyme. GSH is evaluated according to the method of Hissin (Hissin P.J., Hilf R. A fluorometric method for determination of oxydised and reduced Glutathione in tissues. Analytical Biochemistry (1977) vol 74, pp 214-226).

Human fibroblasts were inoculated with growth medium (DMEM+FCS) and incubated 3 days at 37°C, with 5% CO_2. The growth medium was then exchanged with medium containing an ingredient to be tested and incubated 2 days at 37°C with CO2=5%. After an exchange of medium with balanced salt solution, the cell culture was irradiated by UVA 20J/cm². Cell proteins and GSH were measured, and MDA released in the supernatant was determined spectrophotometrically.

Table 1

Results in % against control (mean on 2-3 assays in triplicata):

Product	Dose % w/v	MDA released	Cell proteins	Cell GSH/protein ratio
Control (not irradiated)	-	0	100	100
Control / UVA (20 J/cm2)	-	100	107	78
Rutin *	0.003	79	126	72
	0.01	72	128	71
Rutin octadecandioate according to example 1	0.001	46	139	73
	0.003	15	133	122
Rutin hexadecandioate according to example 2	0.003	46	129	101
	0.01	21	178	75
Dirutin hexadecandioate according to example 10	0.001	39	138	98
	0.003	9	149	154
Mixture of Rutin hexadecandioate and Dirutin hexadecandioate according to example 10	0.001	48	142	92
	0.003	25	143	153
Rutin azelaiate according to example 3	0.001	78	144	67
	0.003	64	165	64
Rutin 11-mercaptoundecanoate according to example 4	0.001	34	94	131
	0.003	0	89	283

*Rutin was purchased from Sigma.

The UVA irradiation has induced a release of MDA and a decrease of cell GSH. After incubation of the fibroblast with esters of rutin, a strong protection of cells against UVA-induced MDA released and GSH decrease was obtained, whereas rutin had very poorly protected the fibroblasts.

Example 9. UVB- cytophotoprotection and anti-inflammatory effect

The arachidonic cascade is an important mechanism of cutaneous inflammation. This cascade may be induced by several factors, particularly by UVB irradiation. UVB induces the inflammatory response by activation of phospholipase A2 (PLA2), which results in a release of arachidonic acid from cell membranes. Then other specific enzymes (so called cyclo-oxygenases) transform arachidonic acid in active components called prostaglandin (PG) which are secreted ~~of~~ from the cells. The fixation of certain prostaglandins (PGE2) on specific skin receptors is followed by redness and swelling on human skin. On cultured human cells, these UVB effects on cell's membrane are associated with a release of a cytoplasmic enzyme into the supernatant medium: Lactate Dehydrogenase or LDH.

Human keratinocytes were inoculated with growth medium (DMEM+FCS) and incubated 3 days at 37°C and 5% CO_2. The growth medium was then exchanged with balanced salt solution containing the ingredient to be tested, the cell culture was irradiated by UVB 50 mJ/cm² (DUKE GL40E lamp). After 1 day of incubation at 37°C with 5% CO2, LDH and PGE2 released in the medium were determined, and cellular DNA was measured using a fluorescent probe to determine the cell viability.

Table 2

Results in % against control (mean on 2-3 assays in triplicata):

Product	Dose % w/v	Keratinocytes DNA	LDH released	PGE2 released
Control (not irradiated)	-	100	0	0
Control / UVB (50mJ/cm2)	-	23	100	100
Rutin *	0.03	69	17	0
	0.1	73	18	10
Rutin octadecandioate according to example 1	0.001	23	73	28
	0.003	49	31	3
Rutin hexadecandioate according to example 2	0.003	24	53	2
	0.01	39	19	0
Dirutin hexadecandioate according to example 10	0.001	38	44	3
	0.003	35	33	1
Mixture of Rutin hexadecandioate and Dirutin hexadecandioate according to example 10	0.0003	36	59	27
	0.001	37	38	1
Rutin azelaiate according to example 3	0.003	51	45	28
	0.01	53	27	19
Rutin 11-mercaptoundecanoate according to example 4	0.0001	44	75	26
	0.0003	41	92	12

*Rutin was purchased from Sigma.

The UVB irradiation has induced an inflammation with a release of PGE2 and with cell membrane injury as demonstrated by the release of LDH activity in the medium, and a decrease of keratinocytes cell number (decrease of around 77% of cell DNA). After incubation of the keratinocytes with rutin or the esters of rutin with ω-substituted fatty acid, and UVB irradiation, an increase of viable cells and a decrease of released LDH and PGE2 was obtained. But the esters of rutin are effective at doses 3-100 times lower than the active doses of rutin. These results demonstrate the anti-inflammatory efficacy of the tested products and their ability to protect cells from the damages induced by the UVB irradiation.

Example 10: Synthesis of diester of rutin with hexadecandioic acid : rutin-C16 diacid -rutin

This reaction was carried out in a 250 ml batch reactor. Rutin (10 g, 16.4 mmol) and hexadecandioic acid (4.2 g, 14.8 mmol) were dissolved in 250 ml *tert*-amyl alcohol. The medium was heated at 80°C under vacuum (400 mbar). The formed vapor was condensed and recycled to the reactor through a column filled with molecular sieves (50 g) overnight. This procedure allowed a low water level (< 100 mM) in the reactor. 7.5 g of the lipase of *Candida antarctica* (Novozym 435) was then added.

After 72 h the enzyme was recovered by filtration. The medium was then concentrated by evaporation of solvent. The medium is a mixture of rutin (10.4%), hexadecandioic acid (6.4%), rutin hexadecandioate (45.1%), dirutin hexadecandioate (38.1 %). The purification by preparative HPLC allowed the separation of rutin hexadecandioate (rutin-O-(C=O)-$(CH_2)_{14}$-COOH) as characterised in example 2, of dirutin hexadecandioate (rutin-O-(C=O-$(CH_2)_{14}$-(C=O)-O-rutin), and of their mixture.

The ^{1}H NMR of the dirutin hexadecandioate obtained was :
^{1}H NMR : (400 MHz, DMSO d_6) : δ 0.75 (d, 6H), 1.2 (m, 22H), 1.43(m, 4H), 2.13 (m, 4H), 3.1-3.7 (broad, 22H), 3.7 (d, 1H), 4.45 (s, 2H), 4.64 (t, 2H), 5.43 (s, 2H), 6.18 (s, 2H), 6.35 (s, 2H), 6.84 (d, 2H), 7.50 (m, 4H), 12.6 (s, 2H, OH) ppm.

Example 11. Solubility in hydrophylic and lipophilic solvent

The solubility ~~were~~ was determined by HPLC measurement after stirring during 1 hour at room temperature.

Table 3.

Product	Solubility in octyl-dodecanol	Solubility in butylene glycol	Solubility in water
Rutin *	0.03 g/L 0.05 mM	22.6 g/L 37.1 mM	0.16 g/L 0.27 mM
Rutin hexadecandioate according to example 2	0.13 g/L 0.15 mM	39.4 g/L 44.7 mM	0.38 g/L 0.43 mM
Dirutin hexadecandioate according to example 10	0.03 g/L 0.02 mM	> 138 g/L 94 mM	0.58 g/L 0.39 mM
Rutin 11-mercaptoundecanoate according to example 4	0.15 g/L 0.19 mM	54.5 g/L 67.2 mM	*not determined*

*Rutin was purchased from Sigma

The derivatives esters of the flavonoids have a higher solubility than the rutin in lipophilic and hydrophilic solvents as octyl-dodecanol, butylene glycol or water.

Example 12. Anti-free radical activity

Free radicals (FR) are reactive chemical species, characterised by non conjugated free electron. FR can appear from unsaturated lipids, certain amino-acids and above all from oxygen during spontaneous biological mechanism such as respiratory chain in mitochondria, or during natural biological process such as inflammation. Oxidative stress like UV or chemical pollutants induces also the rise of free radicals which provokes damages on all cellular and tissue constituents (lipids, proteins, sugars and nucleic bases) of living organisms. Indeed the FR toxicity is deeply enhanced by oxygen level and constitute a key process in ageing, in the appearance of serious diseases such as cancers, diabetes etc. [[...]]

The anti-free radical (anti-FR) activity has been evaluated by biochemical tests to address the potential for scavenging superoxide anion (O2°). The O2° appears mainly from lipoxygenase activity, displayed by leukocytes along the leukotriens synthesis from arachidonic acid released during inflammatory process (Bouclier M & Hensby CN. Prostaglandines et leucotriènes en physiologie cutanée. Bulletin d'Esthétique Dermatologique et de Cosmétologie, (1986) pp 17-22).

Lipoxygenase was incubated with a specific substrate (unsaturated fatty acid) and the flavonoid esters. Then the rate of released superoxide anions was determined using Luminol luminescent probe to calculate the IC_{50} (mean of 2 assays).

Product	IC_{50} (w/v).
Rutin octadecandioate according to example 1	0.0034
Rutin hexadecandioate according to example 2	0.0036
Dirutin hexadecandioate according to example 10	0.0028
Rutin azelaiate according to example 3	0.0025